

Figure E5. Gating strategy for dendritic cell subsets: Live PBMC were gated on by forward vs side scatter. The lineage Lin⁻ 1 (CD3, CD14, CD16, CD19, CD20, CD56)- FITC negative population (Lin⁻) was gated on. Next, the HLA-DR positive population was gated on. Myeloid DC were identified by plotting HLA-DR vs. CD11c. The myeloid DC population was identified as the Lin⁻HLA-DR⁺CD11c⁺ population. Plasmacytoid DC were identified by plotting HLA-DR vs. CD123. Plasmacytoid DC were identified as the Lin⁻HLA-DR⁺CD123⁺ population. Isotype controls were used to determine the background levels. Rectangular gates indicate selected population. Please see representative plots below.

Figure E6. Gating strategy for monocyte subsets: Live PBMC were gated on by forward vs side scatter. The CD14⁺ population (monocytes) was then gated on. The three human peripheral blood monocyte subsets (CD14⁺CD16⁻, CD14⁺CD16⁺ and CD14^{low}CD16⁺) were identified by quadrants on the CD14 vs. CD16 plot. Isotype controls were used to determine the background levels. Please see representative plots below. Rectangular gates indicate selected population.

| | Placebo (n=44) | Vitamin D (n=56) |
|--------------------------|-----------------------|----------------------|
| Age | 42.1 ± 13.4 | 44.0 ± 11.6 |
| Gender | 15 M (33%) 29 F (67%) | 23M (41%) 33 F (59%) |
| Race/Ethnicity | | |
| White | 23 (52%) | 28 (50%) |
| Black | 12 (27%) | 16 (28%) |
| AI/AN | 0 (0%) | 1 (2%) |
| Asian/PI | 2 (4%) | 2 (4%) |
| Hispanic | 7 (16%) | 7 (12%) |
| Other | 0 (0%) | 2 (4%) |
| Asthma years | 25.8 ± 13.3 | 28.1 ± 13.8 |
| Body mass index | 34 ± 10.9 | 33.4 ± 7.9 |
| Vitamin D level at entry | 19.4 ± 6.5 | 19.3 ± 6.3 |

Table E1: Baseline characteristics of randomized participants. Where appropriate, values shown are the mean ± standard deviation.

| | Placebo | | Vitamin D | |
|--------|------------|------------|------------|------------|
| | V3 | V6 | V3 | V6 |
| IL-2 | 1.42 ± 2.2 | 1.47±2.2 | 1.03±2.1 | 1.06±2.0 |
| IL-4 | 0.73±1.0 | 0.77±1.34 | 0.52±1.0 | 0.46±0.8 |
| IL-6 | 1.36±1.9 | 1.87±4.8 | 1.36±2.1 | 0.95±1.5 |
| IL-10 | 0.67±1.0 | 0.77±1.26 | 0.39±0.9 | 0.43±0.9 |
| TNFα | 0.27±0.7 | 0.26±0.7 | 0.19±0.6 | 0.18±0.6 |
| IFNγ | 0.62±1.2 | 0.50±1.0 | 0.33±0.9 | 0.23±0.68 |
| IL-17A | nd | nd | nd | nd |
| TGFβ | 13001±4490 | 13175±4085 | 13183±3829 | 12917±4458 |

Table E2: Serum cytokine levels. Serum was collected prior to (V3) and after (V6) 12 weeks of treatment with vitamin D or placebo. Cytokine levels were measured by cytokine bead array or ELISA (TGFβ only). Presented is the mean ± standard deviation. All results shown are in pg/ml. nd= none detected.

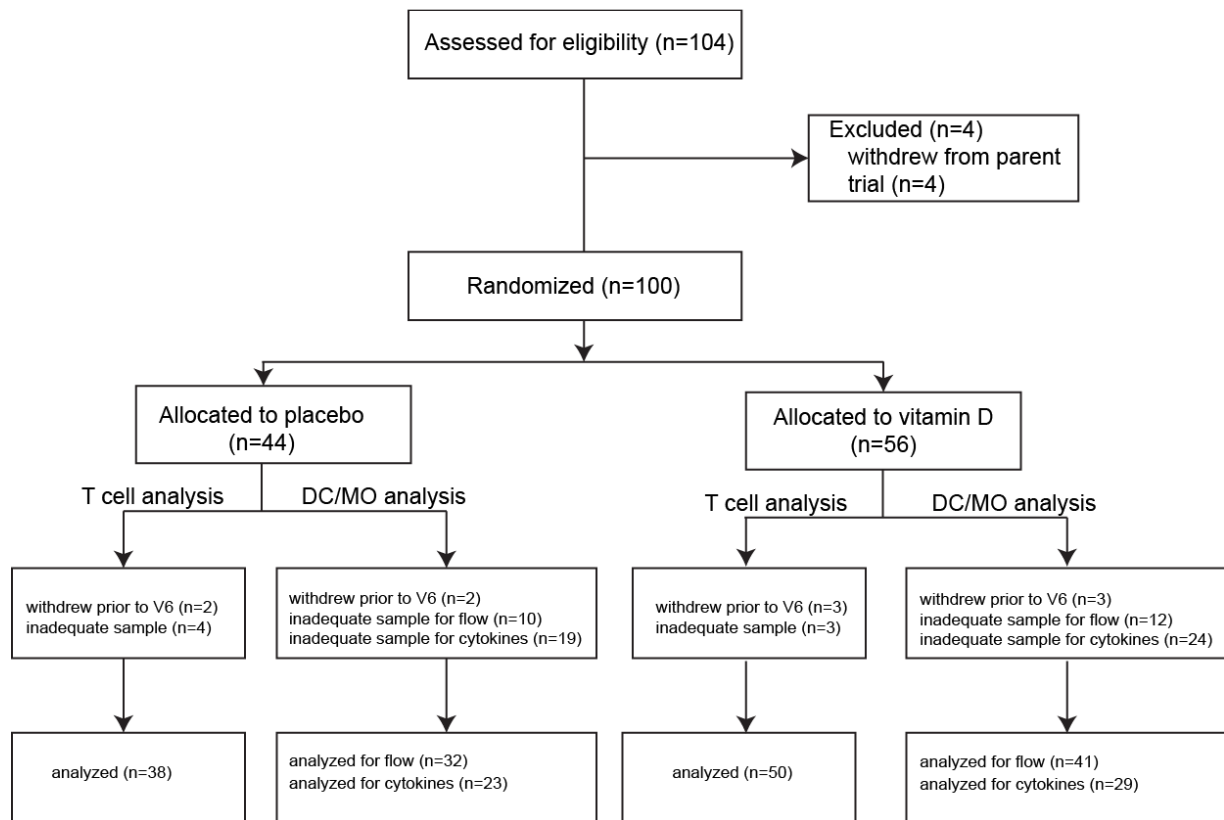


Figure E1: Participant Flow. Participants in the VIDA trial were approached for optional enrollment in this substudy. Samples from the first 100 eligible participants consenting to the study were collected at each participating site and shipped to the research labs at Washington University (T cell studies) or Brigham and Women’s Hospital (myeloid cell studies). As the sample requirements were different between labs, a sample might have been usable at one site but not the other, accounting for the different numbers available for final analysis.

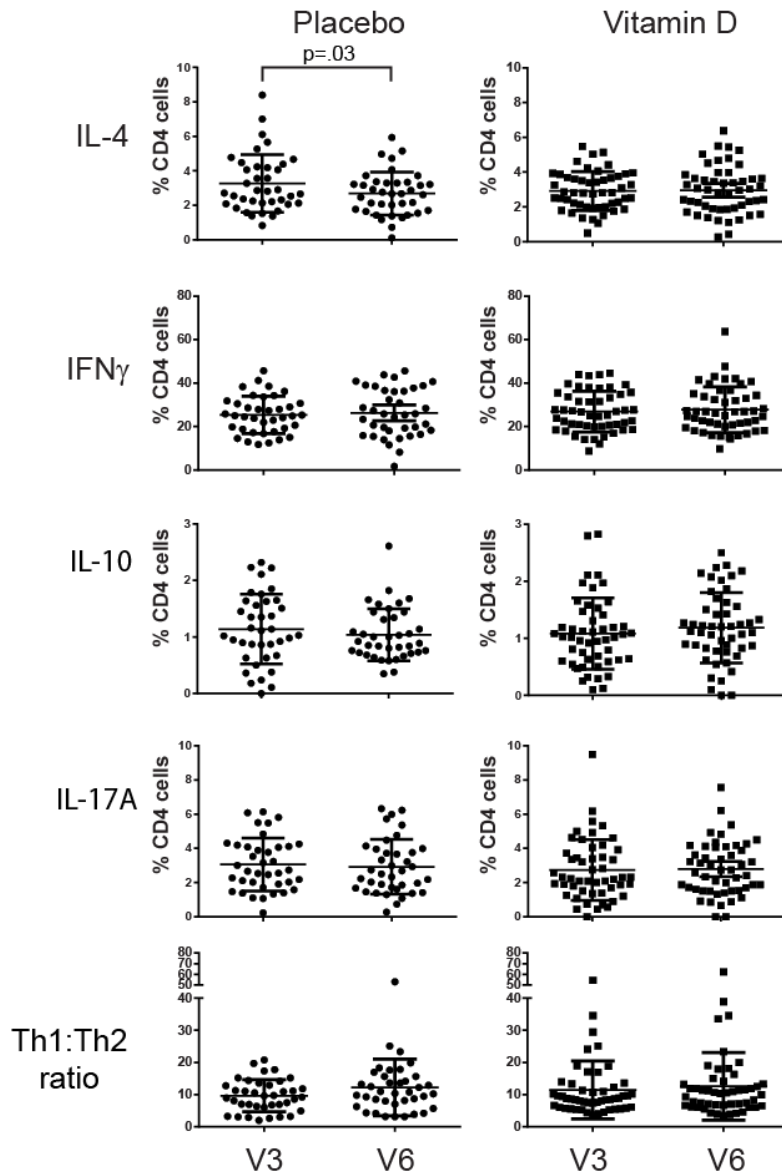


Figure E2: Intracellular cytokine analysis of CD4⁺ T cells. Shown are individual data points for intracellular cytokine staining of CD4⁺ T cells at V3 and V6, which were used to generate the fold change data presented in Figure 1. Samples were obtained prior (V3) to and after (V6) 12 weeks of treatment with placebo or vitamin D as described for Figure 1 in the body of the paper. There was no effect of vitamin D repletion on the percentage of CD4⁺ T cells expressing IL-4, IFN γ , IL-10 or IL-17A. There was a small but statistically significant decrease in the percentage of IL-4 secreting cells in the placebo treated group ($p=.03$), but no change in the ratio of Th1 to Th2 cells, and no difference in the fold change of IL-4 secreting cells from V6 to V3 (see Figure 1). Th1:Th2 ratio was calculated by dividing the percentage of CD4/IFN γ positive by the percentage of CD4/IL-4 positive cells. Data shown are individual sample values as well as the mean \pm standard deviation for the group.

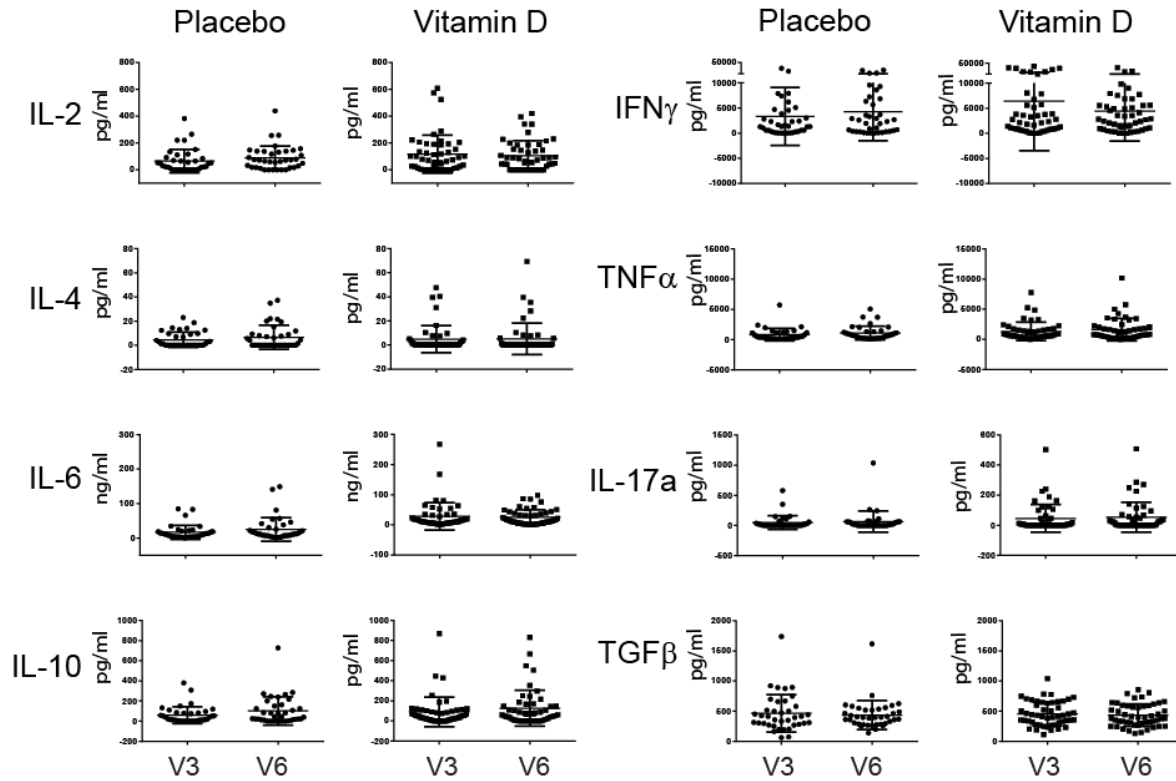


Figure E3: Cytokine secretion of stimulated PBMCs. Shown are the individual values used to determine the fold change in cytokine secretion from V3 to V6 in Figure 2A. PBMC were isolated from samples collected either before (V3) or after (V6) vitamin D supplementation or placebo, and stimulated with α -CD3 and α -CD28 for 48 hours. Secreted cytokines were analyzed in the culture supernatant by cytokine bead array or ELISA (TGF β only). Data shown are individual sample values as well as the mean \pm standard deviation for the group.

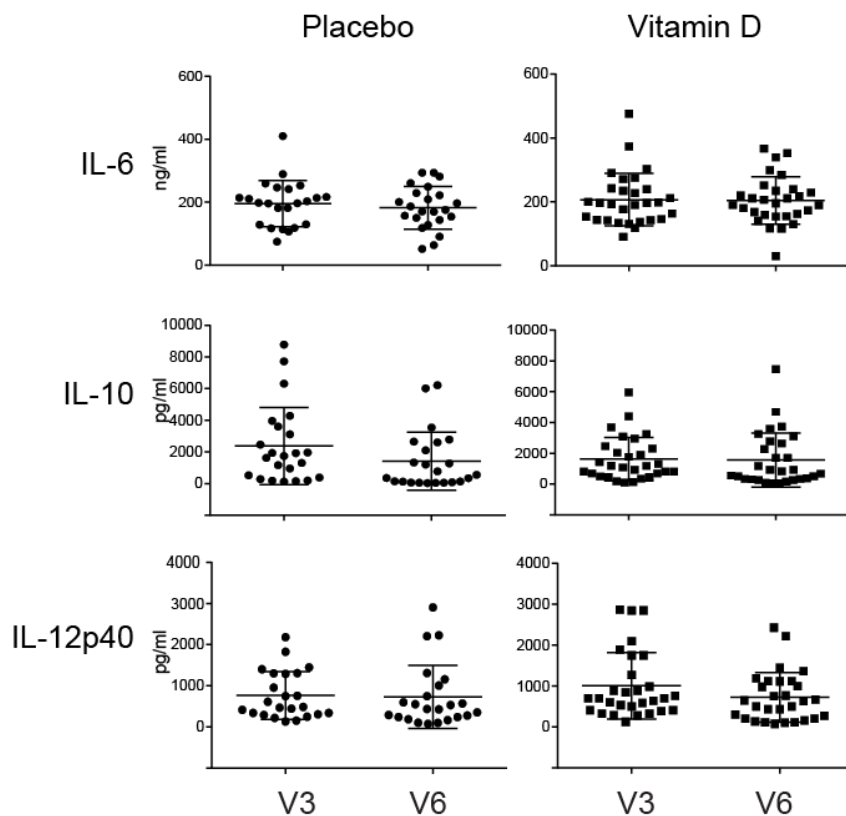


Figure E4. Monocyte cytokine production is not altered by vitamin D supplementation.. Shown are the individual values used to determine the fold change in cytokine secretion from monocytes shown in Figure 2B. CD14⁺ monocytes were isolated from PBMC from vitamin D-deficient asthmatics before (V3) and after (V6) vitamin D supplementation or placebo and stimulated with LPS for 48 hours. IL-6, IL-10 and IL-12p40 production was determined by ELISA. Data shown are individual sample values as well as the mean \pm standard deviation for the group.

